

Validation Viewpoint

During routine use of a validated analytical method, results occasionally will fall outside of the defined specifications. When results fail to meet specifications, an investigation should be triggered so that corrective action can be taken. This column discusses potential sources and ways to avoid out-of-specification (OOS) results and highlights the draft FDA guidelines on OOS investigations.

**Michael Swartz and
Ira Krull**
Validation Viewpoint Editors

Investigating Out-of-Specification Results

It seems to happen in every laboratory sooner or later, no matter how well-validated and robust the method: inevitably, analysts obtain a result that falls outside the specification or acceptance criteria. And while steps can be taken to decrease the frequency of out-of-specification (OOS) results, it is rare that they can be prevented completely. The United States Food and Drug Administration (FDA) regulations require that an investigation be conducted whenever an OOS test result is obtained. Therefore, it is essential in a regulated laboratory to have a standard operating procedure in place that describes the actions to take to determine the cause of the OOS result and the corrective action that must be undertaken. A thorough standard operating procedure will ensure that the correct decisions are made regarding the acceptance or rejection of a batch; batch rejection does not negate the need to perform an investigation. Thorough and systematic investigation of an OOS result not only leads to scientifically sound decisions but also is mandated by law in the Code of Federal Register (CFR) and by the decision in the now infamous case of *U.S. v. Barr Laboratories*. Indeed, FDA guidance is available on the topic of OOS investigations, and although this column will discuss the FDA guidance in some detail, we encourage readers to consult the references for additional details (1). FDA guidance documents are always a good source of information because they are prepared for review staff, establish policies intended to achieve consistency in the FDA's policy and regulatory approach, and establish inspection and enforcement policies and procedures. FDA good guidance practices state that official procedures should be followed when communicating new or different regulatory expectations that are not readily apparent to a broad public audience (2). However, like the recently published FDA guidance on method validation (3,4), guidelines on OOS investigations also have

appeared in draft, not final form. A draft guidance represents FDA's current thinking on a particular topic and opens it up for public comment (5). By issuing draft guidances, the FDA can update guidelines based upon advances in technology and knowledge, changes in regulatory requirements, and policy mandates.

FDA's OOS draft guidance applies to active pharmaceutical ingredients, excipients and other components, and the testing of finished products to the extent that current good manufacturing practices (cGMPs) apply. It discusses how to investigate suspect (or OOS) results including responsibilities, the laboratory phase of the investigation, additional testing that might be necessary, when to go beyond laboratory investigations, and the final evaluation of test results.

An Ounce of Prevention

OOS results can come from laboratory, operator, or process and manufacturing errors. However, the best way to minimize the occurrence of OOS results is to prevent them from happening in the first place, and the best way to do that is to have proper laboratory controls in place. The integrity of laboratory testing and record keeping is of fundamental importance to the FDA in pharmaceutical production and control. Proper laboratory controls must include the following:

- standard operating procedures;
- validated analytical methods;
- properly trained and supervised personnel;
- properly qualified and calibrated instrumentation.

Standard operating procedures are written for many laboratory activities including sampling methods, sample handling, test methods, and calibration and maintenance of instrumentation. They are written to ensure uniformity and are necessary for assuring compliance with regulatory requirements. The standard operating pro-

cedure covering OOS results must define the responsibility for the investigation and provide clear direction to laboratory personnel.

By validating an analytical method, documented evidence is obtained that the method accomplishes or is suitable for its intended purpose. Compendial United States Pharmacopeia (USP) methods need not be validated but simply can be verified for suitability under actual conditions of use. Noncompendial methods must be validated with respect to several parameters including accuracy, precision, linearity, limit of quantitation or detection, robustness, specificity, and range. Both the USP and the International Conference on Harmonization provide guidelines for validating noncompendial methods (4,6–8), and they have been reviewed previously in this column and elsewhere (9–11).

Properly trained and supervised laboratory personnel are needed to effectively perform laboratory operations according to established procedures. Having an adequately trained laboratory staff can cut down on the frequency of retests, investigations, and staff turnover — all of which are red flags for FDA.

Just like a method, an instrument must be suitable for its intended use. Instrument validation, referred to as qualification, is accomplished by performing installation, operational, and performance qualifications, along with documented routine calibrations. By documenting the qualification, calibration, and maintenance procedures and thus demonstrating that the instrument can meet a set of predetermined specifications, one variable (the instrument) can be ruled out in any subsequent investigation. In addition, all analytical methods have system suitability requirements, and systems not meeting these requirements should not be used.

Identifying and Assessing OOS Test Results

So what constitutes an OOS result? For the purpose of this discussion, we can use the FDA guidance definition: “OOS results include all suspect results that fall outside the specification or acceptance criteria established in new drug applications, official compendia, or by the manufacturer” (1). Control charts such as that shown in Figure 1 provide an easy way to inspect for OOS results. Once an OOS result is obtained, an investigation must be launched to determine the cause, and each step of the investigation must be docu-

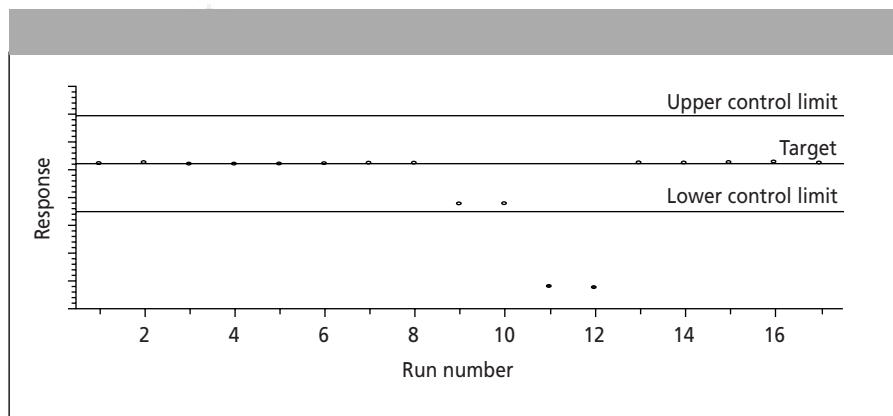


Figure 1: Typical control chart illustrating OOS results. Charts like this can be used to monitor for OOS results. By setting upper and lower control limits around a target or average value according to specifications, OOS samples, injections, and batches can be observed easily.

mented. The first phase of the investigation should include an initial assessment of the accuracy of the data before test solutions are discarded. It is the analyst's responsibility to review the data for compliance with specifications and retain test solutions and inform the supervisor in cases where unexpected results are obtained and no obvious explanation exists. The supervisor's assessment should be objective and timely, and should include the following steps.

- Discuss the test method with the analyst and confirm that the proper procedure was performed.
- Examine the raw data and identify anomalous or suspect information.
- Confirm instrument performance by reviewing qualification and system-suitability data.
- Ensure that proper reference standards, solvents, reagents, and other solutions are used and that they meet quality-control specifications.
- Compare the test-method performance to ensure that it is performing to the standard expected based upon method-validation data.
- Document evidence of this assessment.

Examining retained samples promptly is important to facilitate the assigning of a cause to OOS results. For example, reinjection where a transient instrument malfunction is suspected can provide strong evidence to rule out sample or sample-preparation anomalies. However, laboratory error should be relatively rare. Frequent errors suggest inadequate training, poorly maintained or calibrated instruments, or careless work. Whenever laboratory error is identified, corrective action should be taken to prevent the problem

from reoccurring.

Investigating OOS Test Results

Analysts should not assume that failing test results are attributable to laboratory error without performing and documenting an investigation. If the OOS result cannot be attributed completely to laboratory error, then a full-scale failure investigation must be initiated, with the objective of identifying the source. Varying test results can indicate problems in the manufacturing process or result from sampling problems, and therefore, should be given the highest priority.

General Investigative Principles

The failure investigation should be conducted by the quality-control unit, involving all other departments that can be implicated. It should consist of a timely, thorough, and well-documented review and should include the following steps.

- Clearly identify the reason for the investigation.
- Summarize the manufacturing process sequences that might have caused the problem.
- Provide the results of the documentation review with the assignment of actual or probable cause.
- Determine if the problem has occurred previously.
- Describe any corrective actions taken. Include a list of other batches and products possibly affected, any required corrective actions, and comments and signatures of appropriate personnel.

Laboratory Phase of an Investigation

During the laboratory phase of an investi-

gation, a number of practices are used. These include retesting a portion of the original sample, testing a new specimen from the collection of a new sample from the batch (resampling), and using outlier testing.

Retesting: Sometimes the investigation might involve retesting a portion of the original sample. Retesting often is called for when investigating instrument or sample-handling problems (for example, a suspected dilution error). The retest sample should be taken from the same homogeneous material that originally produced the OOS result. Decisions to retest should be based upon testing objectives and sound scientific judgment and always should be performed by a second analyst (that is, not the one who originally obtained the OOS result). If the OOS result is found to be laboratory error, the retest results are substituted for the original results. The original results must be archived, however, and all explanations must be documented with the proper sign-offs of all involved.

The number of retests should be specified in the standard operating procedure to avoid "testing into compliance," or repeated retesting until a passing result is obtained.

Software that provides for electronic signature sign-off and audit trails helps to maintain regulatory compliance in this regard. If no laboratory or statistical (mathematical) errors can be identified, the original OOS results cannot be invalidated and must be reported along with the retest results.

Resampling: Resampling is different from retesting because it involves analyzing a new specimen from the collection of a new sample from the batch, as opposed to analyzing the original sample. Resampling is used when it is suspected that the original sample was not prepared properly or was not representative of the batch. Resampling should be performed by the same qualified, validated methodology used for the original sample.

Averaging (resampling testing data):

Averaging of test data can be a valid approach, depending upon the sample and its purpose. In some analytical techniques, several discrete measurements often are averaged to report a test result. For example, a high performance liquid chromatography result might be determined by averaging the peak response from replicate injections of the same sample preparation. In this instance, the average result is considered one test and one result. Reliance on averages has the disadvantage of masking

variability among individual test results, however. For this reason, unless averaging is specified by the standard operating procedure, all individual test results should be reported, along with a statistical treatment of the variability. Again, this is common in content-uniformity assays, where the standard deviation is also reported.

Outlier Tests

cGMP regulations require that statistically sound control criteria include acceptance and rejection levels (12). A result might

- 1225), pp. 2256–2259.
(9) I.S. Krull and M.E. Swartz, *LCGC* 15(6), 534 (1997).
(10) I.S. Krull and M.E. Swartz, *LCGC* 15(9), 842 (1997).
(11) M.E. Swartz and I.S. Krull, *Analytical Method Development and Validation, A Primer* (Marcel Dekker, New York, 1997).
(12) *Current Good Manufacturing Practice* (U.S. Food and Drug Administration, Washington, DC, <http://www.fda.gov/cder/dmpq/cgmpregs.htm>).
(13) J.C. Miller and J.N. Miller, *Statistics for Analytical Chemistry* (John Wiley & Sons, Hoboken, New Jersey, 1986).



ADVANSTAR
COMMUNICATIONS

All Rights Reserved. Advanstar Communications Inc. 2003

Summary of statistics for the testing of outliers

Outlier: A value in a set of observations which is so different from the rest that it is considered to be a member of another set or population. The most common method for testing of outliers is probably Dixon's *Q* test (13), which reads as follows:

$$Q \leq \frac{[\text{suspect value} - \text{nearest value}]}{[\text{largest value} - \text{smallest value}]}$$

where [suspect value - nearest value] represents an absolute difference and no sign is intended (1/2). The suspect value is always the number that is farthest from the mean or average value of the set. Critical values for *Q* for any probability value ($P \leq 0.05$ and $P \leq 0.01$) can be found in various tables (13).

For example: The following values were obtained for the nitrite concentration (milligrams per liter) in a sample of river water: 0.403, 0.410, 0.401, 0.380. The last measurement is suspect: should it be entirely rejected?

We have:

$$Q \leq \frac{[0.380 - 0.401]}{[0.410 - 0.380]} = \frac{0.021}{0.030} = 0.7$$

From any table of *Q* values, the critical value for *Q* is 0.831 ($P \leq 0.05$). Because the calculated value of *Q* does not exceed this, the suspect measurement should be retained (*Adapted from reference 13).

qualify as a statistical outlier if it is markedly different from the others in a series of results obtained by a validated method. The use of an outlier test should be determined in advance and again, should be documented in the standard operating procedure. In addition, it should specify the minimum number of results required to obtain a statistically significant assessment. Because an outlier test is only a statistical analysis, it cannot be used to invalidate the data. However, it is useful for the evaluation of the significance of the result for batch evaluations. One note of caution: in cases where the variability of the product is what is being measured (that is, content uniformity), an outlier test should not be applied, because a measurement thought to be an outlier might in fact be an accurate result. The sidebar provides an example of outlier testing.

Concluding the Investigation

Now it is decision time. To conclude the investigation, following the standard operating procedure, the results should be eval-

uated, the batch quality determined, and a release decision should be made. The goal of the investigation is to arrive at one of two conclusions. Either the batch fails and should be rejected (that is, the OOS result is confirmed), or the OOS result is invalidated and a cause is revealed. The OOS result can be invalidated only upon the observation and documentation of a test result that reasonably can be determined to have caused the OOS result. If the OOS result is confirmed, the batch is rejected.

Of course, there is one other possible outcome. Despite all of the controls in place, assessing, identifying, and investigating results still might be inconclusive. In cases where an investigation does not reveal a cause or confirm the OOS result, the OOS result should be retained in the record and taken into account in the batch or lot disposition decision.

Finally, for those products that are the subject of applications, regulations require submitting a field-alert report within three working days concerning any failure of a batch to meet any of the specifications established in an application. As the saying goes, no job is finished until the paperwork is done.

References

- (1) *Guidance for Industry, Investigating Out of Specification (OOS) Test Results for Pharmaceutical Production* (U.S. Food and Drug Administration, Department of Health and Human Services, Rockville, Maryland, September 1998).
- (2) 21 CFR 10.115, *Federal Register*, 65(182), 19 September 2000, 56,468–56,480.
- (3) "Analytical Procedures and Methods Validation," *Federal Register*, 65(169), 30 August 2000, 52,776–52,777.
- (4) R. Brown, M. Caphart, P. Faustino, R. Frankewich, J. Gibbs, E. Leutzinger, G. Lunn, L. Ng, R. Rajogopalan, Y. Chui, and E. Sheinin, *LCGC*, 19(1), 74–79, (2001).
- (5) Pharmaceutical Research and Manufacturers of America, <http://srpub.phrma.org/letters/12.14.98.oos.commentary.html> (1998).
- (6) ICH Q2A: Text on Validation of Analytical Procedures (International Conference on Harmonization of Technical Requirements for the Registration of Drugs For Human Use, Geneva, Switzerland, March 1995). Published in *Federal Register*, 60, 1 March 1995, p. 11,260. Or see: <http://www.ich.org/ich5g.html#Analytical>.
- (7) ICH Q2B: Text on Validation of Analytical Procedures: Methodology (International Conference on Harmonization of Technical Requirements for the Registration of Drugs For Human Use, Geneva, Switzerland, March 1997). Published in *Federal Register*, 62(96), 19 May 1997, 27,463–27,467. Or see: <http://www.ich.org/ich5g.html#Analytical>.
- (8) *The United States Pharmacopeia 25, National Formulary 20*, (The United States Pharmacopoeial Convention, Rockville, Maryland, 2002, Ch.

Michael E. Swartz

"Validation Viewpoint" Co-Editor
Michael E. Swartz is a Principal Scientist at Waters Corp., Milford, Massachusetts, and a member of LCGC's editorial advisory board.



Ira S. Krull

"Validation Viewpoint" Co-Editor
Ira S. Krull is an Associate Professor of chemistry at Northeastern University, Boston, Massachusetts, and a member of LCGC's editorial advisory board.



The columnists regret that time constraints prevent them from responding to individual reader queries. However, readers are welcome to submit specific questions and problems, which the columnists may address in future columns. Direct correspondence about this column to "Validation Viewpoint," LCGC, Woodbridge Corporate Plaza, 485 Route 1 South, Building F, First Floor, Iselin, NJ 08830, e-mail lcgcedit@lcgcmag.com.